



ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating 2017: 3.53
JMPS 2017; 5(4): 31-35
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Received: 18-05-2017
Accepted: 20-06-2017

Phyu Phyu Myint
Department of Chemistry,
Sittway University Sittway,
MYANMAR Myanmar

Aye Nyein Sann
Department of Chemistry,
Sittway University Sittway,
MYANMAR Myanmar

Naing Mar Soe
Department of Chemistry,
Sittway University Sittway,
MYANMAR Myanmar

Screening of phytochemicals, antioxidant and antimicrobial activities of *Blumea lacera* (Burm. f.) DC. Leaf and Root

Phyu Phyu Myint, Aye Nyein Sann and Naing Mar Soe

Abstract

The aim of this study was to examine the possible phytochemicals, antioxidant and anti-microbial activities of *Blumea lacera* (Burm. f.) DC. plant, well known as folk dietary supplement in Rakhine coast, Myanmar. In this study, preliminary phytochemical investigation, DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging and agar well diffusion methods were conducted. The results showed that the presence of steroids, terpenoids, glycosides, phenolic compounds, alkaloids, flavonoids and tannins in samples. Nutrient value has been found as 6.1 and 6.0 % of moisture, 3.6 and 14.2 % of crude fat, 18.4 and 6.0 % of protein, 5.7 and 30.0 % of dietary fiber, 59.7 and 33.2 % of carbohydrate, 6.6 and 10.6 % ash, and 344.8 and 284.6 kcal /100 g based on dried leaf and root samples. *In vitro* antioxidant assay, the result suggested the order of the extracts of EtOH (root) > aqueous (root) > aqueous (leaf) > EtOH (leaf). The antimicrobial activities (*in vitro*) of pet-ether, EtOAc, CH₂Cl₂, 95 % EtOH, MeOH and aqueous extracts were screened on six species of microorganisms, namely *Bacillus pumilus*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Among the tested crude extracts of leaf sample, pet-ether and EtOH extracts were found to possess the most potent antimicrobial activity against all the test microorganisms. However, pet-ether and aqueous extracts did not show the antimicrobial activity for the root sample. From the results of this study, the plant could be applied as the local health remedy to the local indigenous communities of our country.

Keywords: phytoconstituents, antioxidant, *Blumea lacera*, leaf and root, crude extracts, antimicrobial activity

1. Introduction

Blumea lacera (Burm. f.) DC. belongs to family Asteraceae, is a wild plant that grown in the Rakhine Coastal zone in Myanmar. Although the plant is widely used as a dietary supplement in this area, very little scientific evidence was reported on its chemical constituents and biological activities. *Blumea lacera* is an annual herb, with a strong odour of turpentine. Stem is erect, ash colored, densely glandular, pubescent. Fruits are an achene, oblong and not ribbed (Agharkar, 1991) [1]. The leaves are elliptical-oblong to oblance-shaped or obovate (Alonzo, 1999) [3]. The plant occurs throughout the plains of India from the north-west ascending to 2,000 ft in the Himalayas. It is a common roadside weed in Ceylon, Myanmar and Malaya. It is distributed to the Malay Islands, Australia, China and Tropical Africa (Caius, 1986).

Blumea lacera is used in folk medicine for the treatment of cough, bronchitis, dysentery, wound healing. Plants, plant parts, plant products of all descriptions, particularly those with medicinal properties are invariably used as principal components or ingredients of various traditional medicines. Fresh leaves of *Blumea* are the most valuable part. Cancer and atherosclerosis, two major causes of death, are salient "free radical" diseases in human (Bugchi and Puri, 1998) [5]. Free radical result in number of human degenerative diseases affecting a wide variety of physiological functions such as atherosclerosis, diabetes, ischemia/reperfusion (I/R) injury, inflammatory diseases (rheumatoid arthritis, inflammatory bowel diseases and pancreatitis), cancer, neurological diseases, hypertension etc. (Cui, *et. al.*, 2004) [8].

Free radical scavenger or antioxidant may play a major role in the prevention of a number of diseases including cardiovascular and cerebrovascular diseases, some forms of cancer, and may age related disorders (Packer, *et al.*, 2001) [15]. Naturally occurring antioxidants of high or low molecular weight can differ in their composition, their physical and chemical properties and in their mechanism and site of action. Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively

Correspondence

Phyu Phyu Myint
Department of Chemistry,
Sittway University Sittway,
MYANMAR Myanmar

studied, particularly as treatments for stroke and neurodegenerative diseases. However, it is unknown whether oxidative stress is the cause or the consequence of disease (Bjelakovic, *et al.*, 2007) [6].

Medicinal plants are used on a large scale in medicine against drug-resistant bacteria, which are considered one of the most important reasons for the lack of success of treatment in infectious diseases (Almariri, 2014) [2]. The antimicrobial activity of *B. lacera* was reported against some bacteria and fungus (Jahanm *et al.*, 2014) [14]. In this study, 6 strains of microbial species including *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis*, *Candida albicans* and *Escherichia coli* were used for the antimicrobial activity screening. *Candida* yeast can cause uncomfortable symptoms such as vaginitis, weight retention, bowel disorders, ear and sinus irritation, intense itching, canker sores and ring worm (Edwin, 1974) [9]. *Pseudomonas aeruginosa* is an opportunistic pathogen. The bacteria takes advantages of an individual weakened immune system to create an infection and this organism also produce tissue-damaging toxin. Because *B. subtilis* is ubiquitous, it has developed adaptive strategies to subsist in diverse environments via the production and secretion of a large number of genetically encoded molecules that control the growth of neighbouring organisms (Liu, *et al.*, 2010) [10]. Most *E. coli* strains are harmless, but some serotypes can cause serious food

poisoning in humans, and are occasionally responsible for food product recalls due to food contamination (Ishii and Sadawsky, 2008) [13]. The plant contains potential antibacterial components that may be useful for evolution of pharmaceutical for the therapy of ailments.

The development of the use of traditional medicinal plants and traditional medicinal formulation are also one of the important phases in the national health policy. One of the prerequisite for the success of primary health case is the availability and use of suitable drugs. The present study focused on the investigation of chemical constituents, antioxidant and antimicrobial activities of leaf and root of *Blumea lacera* from Myanmar which has not been investigated scientifically.

Materials and Methods

Sample Collection and Preparation

The leaf and root sample of *Blumea lacera* (Burm. f.) DC. (Figure 1) was collected from Mrauk U Township, Rakhine State, Myanmar in June, 2016. The fresh samples were cleaned by washing with water and air-dried. The dried samples were grounded using grinding machine. And then these powdered samples were kept in the sealed air-tight containers to prevent moisture changes and other contamination. It was then used without further purification or refining.



Fig 1: Photograph of *Blumea lacera* (Burm.f)DC. tree and its parts

Determination of Nutritional Values and Phytoconstituents

The amount of nutrients such as moisture, ash, fat, carbohydrate, fiber, protein, vitamins, and calorie contents in the sample were determined by recommended analytical methods (AOAC, 1990) [4]. Preliminary phytochemical investigation was carried out according to the standard procedures (Trease and Evans, 1980) [16], (Robinson, 1983) [12], (Marini-Bettolo, *et al.*, 1981) [11].

Test for alkaloids

Dried powdered sample (3 g) was boiled with 50 mL of 1% hydrochloric acid for about 10 min and allowed to cool and then filtered. The filtrate was divided into four portions and tested separately with Mayer's reagent, Dragendorff's reagent, Wagner's reagent and Sodium picrate solution. Observation was made to see the coloured precipitates, indicating the presence of alkaloids.

Test for flavonoids

Dried powdered sample (3 g) was soaked in 50 mL of ethanol for about 6 h and filtered. A piece of magnesium turning and

a few drops of concentrated hydrochloric acid were added into 5 mL of ethanol extract to see if pink colour appeared, indicating the presence of flavonoids.

Test for glycosides

About 3 g of powdered sample was soaked in 50 mL of ethanol for 6 h and filtered. 5 mL of filtrate were taken and treated with a few drops of 10% lead acetate solution. If white precipitate were formed, it was noticed as the presence of glycosides.

Test for phenolic compounds

Dried powdered sample (3 g) was soaked in 50 mL of ethanol for 6h and filtered. 5 mL of filtrate were taken and treated with a few drops of freshly prepared 1:1 mixture of 1% potassium ferricyanide and 1% ferric chloride solution. The change of colour of solution indicated the presence of phenolic compounds.

Test for tannins

Dried powdered sample (3 g) was boiled with 50 mL of distilled water for about 10 min and filtered. 5 mL of water

extract were taken and treated with a few drops of gelatin and 1% FeCl₃. Observation was made to see precipitates were formed; then the presence of tannins.

Test for steroids

Dried powdered sample (3 g) was soaked in 50 mL of petroleum ether (60-80 °C) for about 6 h and filtered. 3 drops of acetic anhydride and 1 drop of concentrated sulphuric acid were added to 5 mL of petroleum ether extract and recorded the observed colour. If the colour was observed to change blue or greenish blue or green, the steroids were present.

Test for terpenoids

Dried powdered sample (3 g) was soaked in 50 mL of chloroform for about 6 h and filtered. 3 drops of acetic anhydride and one drop of concentrated sulphuric acid were added to 5 mL of chloroform extract and recorded the observed colour. Red or pink colouration indicated the presence of terpenoids.

Test for cyanogenic glycosides

Dried powdered sample (ca. 1 g) was mixed with 50 mL of distilled water in boiling tube. Then about 5 drops of concentrated sulphuric acid was added and sodium picrate paper was trapped in the neck of the test tube by means of a cork. The resulting mixture was heated by using a spirit burner. Observation was made to see if the paper turned brick red which indicated the presence of cyanogenic glycosides.

Preparation of various extracts from plant samples

30 g each of the dried powdered sample was separately percolated with 100 mL of pet- ether (60-80 °C), dichloromethane, ethyl acetate, 95 % ethanol and methanol for one week and filtered. This procedure was repeated for three times. The combined filtrates were concentrated by rotary evaporator to get respective soluble extracts. In addition, aqueous extracts of each sample were prepared by boiling 30g of sample with 100 mL of distilled water for six hours and filtered. It was repeated three times and the filtrates were combined followed by removal of the water to give aqueous extract. Each extract was dried at desiccator under vacuum and stored in refrigerator for the screening of biological activities.

Screening of antioxidant activity of crude extracts from *B. lacera* leaf and root

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of leaf and root materials. This assay has been widely used to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenols in food system (Leea, 2002). In this experiment, the antioxidant activity was studied on 95% ethanol extract, and aqueous extracts from selected samples by DPPH free radical scavenging assay.

DPPH radical scavenging activity was determined by UV spectrophotometric method. The control solution was prepared by mixing 1.5 mL of 60 µM DPPH solution and 1.5 mL of 95

% ethanol using shaker. The sample solution was also prepared by mixing thoroughly 1.5 mL of 60 µM DPPH solution and 1.5 mL of test sample solution. The solutions were allowed to stand at room temperature for 30 minutes. After 30 minutes, the absorbance of these solutions was measured at 517 nm by using UV spectrophotometer. Absorbance measurements were done in triplicate for each solution and then mean values so obtained were used to calculate percent inhibition of oxidation by the equation and then IC₅₀ (50% inhibitory concentration) value were also calculated by linear regressive excel program.

In vitro screening of antimicrobial activity

The antimicrobial activities of different crude extracts such as pet-ether, dichloromethane, ethyl acetate, 95% ethanol, methanol and aqueous extracts were determined against six microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *E.coli* species by employing agar well diffusion method. The extract (1 g) was introduced into sterile petridish and dissolved in 1mL or with least amount of its respective solvent till it was dissolved. Trypticase soy broth (30 g) was suspended in 100 mL of distilled water in a conical flask, covered with aluminium foil and mixed thoroughly and heated to completely dissolve on a hot plate stirrer and sterilized by autoclaving for 15 minutes at 121 °C. The bacteria suspension from tripticase soy broth was done evenly onto the surface of the tripticase soy agar plates immediately after hardening of the agar well were made with a 10 mm sterile cork bore from each seeded agar. After inoculums had been dried for 5 minutes, the agar disc was removed and the wells were filled with sample to be tested. And then, the plates were incubated at 37 °C. After overnight incubation at 37 °C, the diameter of inhibition zone including 10 mm wells were measured. The well plate dilution method was used to test antimicrobial action of the extracts on 24 hours broth culture of the organism used. The extracts from the sample were tested with six microorganisms.

Results and Discussion

Some nutritional Values of Plants samples

The nutritional values such as protein, fiber, fat and carbohydrates of *B. lacera* leaf and root samples were also determined. The moisture content of samples was determined by AOAC method and was found 6.1 and 6.0 %. The total ash in the sample is the inorganic residue remaining after the organic matter has been burnt away and it was found 6.6 and 10.6 %. The fat content was determined by the soxhlet extraction method and 3.6 and 14.2 % was obtained. In addition, the sample was also studied for fiber content by Fiber Cap method, protein content by AOAC method and ash content by using muffle furnace. The crude fiber and protein contents for *B. lacera* leaf and root were found to be 5.7 and 30.0 % and 18.4 and 6.0 %, respectively. And 59.7 and 33.2 % of carbohydrate was observed to be present in the leaf and root samples and energy value was found to be 344.8 and 284.6 kcal/100g (Figure 2).

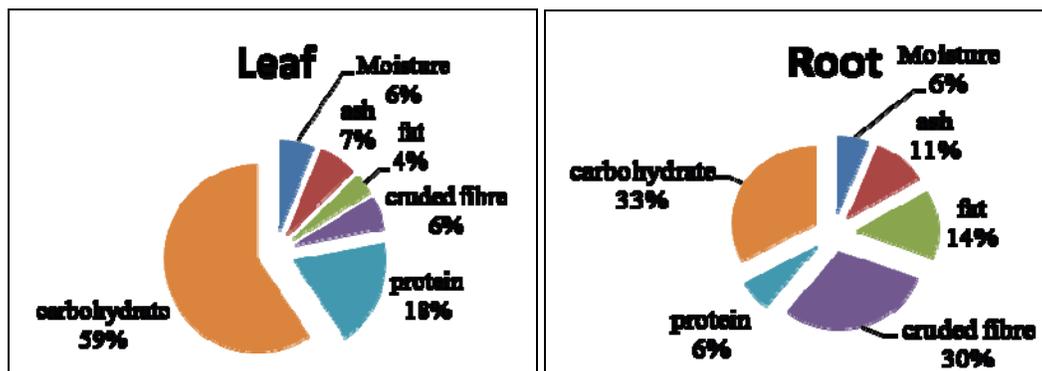


Fig 2: Nutrients value of *Blumea lacera* leaf and root

Physicochemical Constituents of Plants Samples

Preliminary phytochemical analyses were performed in order to know different types of chemical constituents present in *B. lacera* leaf and root. Phytochemical results are summarized in Table 1. It was investigated that steroids, terpenoids, glycosides, phenolic compounds, flavonoids and tannins were

found to be present in leaf and root of *Blumea lacera*. However, cyanogenic glycoside was not observed in both samples and alkaloids could not be found in root sample. On the basis of phytochemical analyses, many bioactive constituents present in leaf and root of the sample and may recover the disorder of diseases.

Table 1: Results of Phytochemical Investigation on *Blumea lacera* Leaf and Root

Sr. No.	Tests	Extract	Test Reagents	Observation	Leaf	Root
1	Alkaloids	1% HCl	Mayer's reagent Dragendorff's reagent Wagner's reagent Sodium picrate	white ppt orange ppt brown ppt yellow ppt	-	+
2	Flavonoids	EtOH	Mg turning & conc.HCl	pink colour	+	+
3	Glycosides	EtOH	10% lead acetate	white ppt	+	+
4	Phenolic compounds	EtOH	10 % FeCl ₃	Deep blue colour	+	+
5	Tannins	H ₂ O	1 %Gelatin & 2% NaCl	white ppt	+	+
6	Steroids	Pet-ether	Acetic anhydride & conc. H ₂ SO ₄	green colour	+	+
7	Terpenoids	CHCl ₃	Acetic anhydride & conc. H ₂ SO ₄	pink colour	+	+
8	Cyanogenic glycosides	H ₂ O	Sodium picrate paper	no brick red	-	-

(+) presence, (-) absence

Antioxidant Activity of Crude Extracts from *Blumea lacera* Leaf and Root

The antioxidant activity was studied on the 95% ethanol and aqueous extracts from *B. lacera* leaf and root by DPPH free radical scavenging assay method. DPPH (1,1-diphenyl -2-picrylhydrazyl) method is the most widely reported method for screening of antioxidant activity on many plant drugs. This method is based on the reduction of coloured free radical DPPH in ethanolic solution by different concentration of the samples. The antioxidant activity was expressed as 50% oxidative inhibitory concentration (IC₅₀).

The present study was carried out to investigate the radical scavenging activity of the leaf and root sample using two crude extracts such as ethanol and aqueous extracts according to the spectrophotometric method. In this experiment, various different concentrations (0.16 -100 µg/mL) of each crude extract of leaf and root samples were prepared in ethanol solvent. Ascorbic acid was used as standard and ethanol without crude extract was employed as control. Determination of absorbance was carried out at wave length 517 nm using UV visible spectrophotometer. Each experiment was done triplicate.

From these experimental results, it was found that as the concentrations increased, the absorbance values decreased i.e. increase in radical scavenging activity of crude extracts usually expressed in term of % inhibition. From the average values of % inhibition, IC₅₀ (50% inhibition concentration) values in µg/mL were calculated by linear regressive excel program.

The IC₅₀ values were found to be 37.04 and 11.42 µg/mL for ethanol extract and 33.49 and 30.07 µg/mL for aqueous extract of the leaf and root samples. Among these extracts, since the lower the IC₅₀ showed the higher the free radical scavenging activity. Although all of these extracts have the lower antioxidant activity than standard ascorbic acid (IC₅₀=1.90 µg/mL), ethanol extract was found to be more effective than aqueous extract in free radical scavenging activity in root sample, however, aqueous extract was found to be slightly more potent in leaf sample. The present study proof that *Blumea lacera* (Burm. f) DC root is having favourable antioxidant activity and is suitable to develop a drug for the prevention of human disease related to free radical mechanism.

Antimicrobial Activity of *Blumea lacera* (Burm.f) DC. Leaf and Root Samples

Screening of antimicrobial activity of crude extracts such as Pet-ether, dichloromethane, EtOAc, EtOH, MeOH and H₂O extracts from *B. lacera* root samples was done by agar well diffusion method. In this investigation, the extracts were tested against six microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *E.coli* species. The measurable zone diameter showed the degree of antimicrobial activity.

It was observed that all extracts of *B. lacera* leaf except water extract exhibited inhibition zone diameters between 12-14 mm against *Pseudomonas aeruginosa* species of

microorganism tested. Pet-ether, CH_2Cl_2 and EtOH extract of *B. lacera* leaf showed antimicrobial activity against *Bacillus pumilus* species ranging the inhibition zone diameter 13-15 mm. PE, MeOH and EtOH extract of *B. lacera* leaf showed inhibition zone diameters ranging 13-15 mm against *Staphylococcus aureus*, *Pseudomona aeruginosa*, and *Candida albican*. All extracts except water extract exhibited inhibition zone diameters between 12-15 mm against of microorganism tested. Therefore these extracts may have mild broad spectrum activity.

For the *B. lacera* root sample, it was exhibited inhibition zone

diameters of 12 mm against *Pseudomonas aeruginosa* species of microorganism tested. CH_2Cl_2 , EtOAc, EtOH and MeOH extracts of *B. lacera* root showed antimicrobial activity against all tested microorganisms except *Pseudomonas aeruginosa* ranging the inhibition zone diameter 11-15 mm. However, Pet-ether and aqueous extracts of *B. lacera* root did not show inhibition zone diameters for tested microorganisms. Therefore these extracts except pet ether and aqueous extracts may have mild broad spectrum activity and would be helpful in testing diseases caused by infection of six species microorganisms.

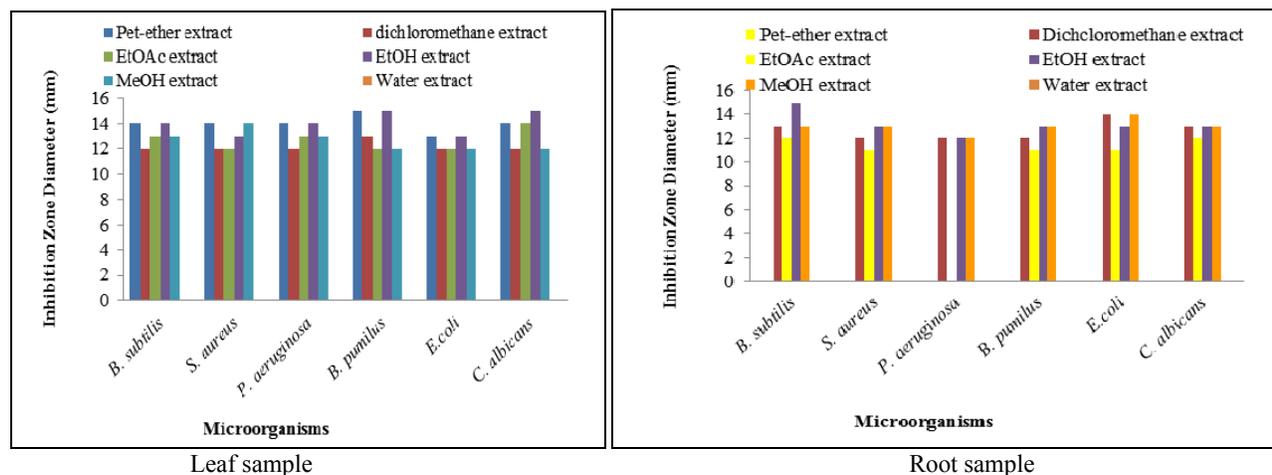


Fig 3: Comparison of inhibition zone diameters for various crude extracts against six species of microorganisms

Conclusion

The results suggest that reactive free radicals can be scavenged through chemoprevention utilizing natural antioxidant compounds present in this locally available medicinal plant. The ethanolic and aqueous extracts of *Blumea lacera* leaf and root samples showed potent antioxidant and antimicrobial activities and it can be used for the drug discovery development and nutrient-rich food supplement. This plant derived medicines offer potential for cost effective management oxidative stress and related diseases through dietary interventions, nutrient supplementation, and combination therapies with sole medication from natural sources over the long term. Although the structure of active agents obtained from plants have not been well characterized, the presences of natural antioxidants (tannins, flavonoids, phenolic compounds) are mainly responsible to prevent reactive free radical formation and proliferation of microorganisms.

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